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(54) Title: DEVICE HAVING OSTEOINDUCTIVE AND OSTEOCONDUCTIVE PROPERTIES

(57) Abstract: The present invention relates to a device having osteoinductive and osteoconductive properties in vivo comprising a carrier containing calcium phosphate and an osteoinductive protein, wherein said carrier is homogenously coated with said protein. Moreover, the present invention relates to a method for the production of a device having osteoinductive and osteoconductive properties in vivo. The invention encompasses a pharmaceutical composition comprising the device of the invention or a device which is obtainable by the method of the invention and relates to the use of said device for the preparation of a pharmaceutical composition to be used for bone augmentation, for treating bone defects, for treating degenerative and traumatic disc disease, for treating bone dehiscence or to be used for sinus floor elevation. Finally, the invention relates to a kit comprising the device of the invention or a device which is obtainable by the method of the invention.

Device having osteoinductive and osteoconductive properties

The present invention relates to a device having osteoinductive and osteoconductive properties in vivo comprising a carrier containing calcium phosphate and an osteoinductive protein, wherein said carrier is homogeneously coated with said protein. Moreover, the present invention relates to a method for the production of a device having osteoinductive and osteoconductive properties in vivo. The invention encompasses a pharmaceutical composition comprising the device of the invention or a device which is obtainable by the method of the invention and relates to the use of said device for the preparation of a pharmaceutical composition to be used for bone augmentation, for treating bone defects, for treating degenerative and traumatic disc disease, for sinus floor elevation and for treatment of bone dehiscence. Finally, the invention relates to a kit comprising the device of the invention or a device which is obtainable by the method of the invention.

Various calcium phosphates such as beta-tricalcium phosphate ($Ca_3(PO_4)_2$) (beta-TCP), alpha-tricalcium phosphate (alpha-TCP) and hydroxy apatite (HA) have been shown to be effective as bone replacement materials. Beta-TCP, for example, is suitable both as granulate and in pieces (blocks) for the treatment of bone defects. The bone replacements materials containing calcium phosphate are usually used when the regeneration of the bone is not possible any more or is possible with difficulties only. In addition, bone replacement materials are used when the formation of additional bone is a prerequisite for a subsequent setting of an implant. The calcium phosphates exhibit an osteoconductive effect, i.e. they represent an inert structure facilitating the migration of cells from the neighbouring bone. The presence of bones or different mesenchymal cells, however, is a precondition for the new formation of bones. The effect of calcium phosphates can be significantly increased by adding bone chips. The bones are not only osteoconductive but also osteogenic

(stimulation of bone cells for the neosynthesis of bone material) and osteoinductive, i.e. they cause the transformation of undifferentiated mesenchymal stem cells in osteoblasts and chondrocytes. For reasons of safety, autogenic bone chips are preferred to the allogenic or xenogenic preparations. The production of autogenic bones, however, always involves a second surgical procedure, which, in many cases, is not accepted by the patient.

An alternative to the use of autogenic bones is the use of specific bone growth and differentiation factors such as GDF-5 or different bone morphogenetic proteins (BMPs). These protein factors have an osteoinductive effect which, however, they can only exert if they are used in an immobilized form. In the literature, both calcium phosphates, collagen and mineralised collagen (collagen-containing calcium phosphate) are described as carriers (hydroxy apatite and beta-TCP (Hotz, 1994), hydroxylic apatite from algae extracts (Gao, 1996), bone extracts (Gombotz, 1996) and collagen (Friess, 1999). The analyses of the potency of the coated carriers, which are described in the literature, do not present a uniform picture but exhibit significant variations which are a consequence of either the carrier type selected or the coating method (Terheyden et al. (1997)). Various methods are described. In WO 98/21972 coating is achieved by rapid precipitation of GDF-5 onto beta-TCP is achieved by first dissolving GDF-5 in an organic solvent and then precipitating it by adding water. Due to the toxicity of many solvents, however, such a process is not preferred for the production of pharmaceutical compositions. Lind et al. (1996) carry out the coating of various calcium phosphate ceramics in the presence of gelatine (usually obtained from bovine or pig bones) as protection protein. Due to the increased risk of infection, however, the use of animal substances should be avoided for the production of pharmaceutical compositions and medicinal products. Friess et al. (1999) and Gao et al. (1996) describe the coating of collagens with BMP-2. Due to the low compressive strength of collagens, such carriers, however, are not suitable for all indications. This particularly applies to indications with which the newly-formed bone has to sustain a later pressure load. Furthermore, pharmaceutical qualities of collagen are so far available from animal sources only.

Although several papers describe the use of protein coated carriers for bone augmentation, efficient and reliable methods for manufacturing and use for treating

bone defects are not available but nevertheless highly desirable.

Thus, the technical problem underlying the present invention is to provide means and methods for efficiently and reliably treating bone defects comprising bone augmentation.
The technical problem is solved by the embodiments characterized in the claims.

Accordingly, the present invention relates to a device having osteoinductive and osteoconductive properties in vivo comprising a carrier containing calcium phosphate and an osteoinductive protein, wherein said carrier is homogeneously coated with said protein.

The term "device" as used in accordance to the present invention refers to a entity which comprises at least two components. One of said components is a carrier matrix. Preferably, said carrier matrix consists of inorganic ceramics. Said ceramics have a particularly high surface due to the presence of macro- and micro pores. Preferably, said macro- pores have a diameter of approximately 100 to 400 nm while the micro- pores have a diameter of less than 10 nm. Most preferably, said carrier is a calcium phosphate as referred to infra.
Another component of said device is a protein or polypeptide which has osteoinductive properties as will be explained in detail below. The protein or polypeptide is immobilized on the surface of the carrier. The osteoinductive proteins and polypeptides applied in accordance with the present invention have a particular high affinity for inorganic carrier matrices such as calcium phosphate. Preferably, the binding of said protein or polypeptide to the carrier is reversible. Thereby, dissolution of said protein is allowed once the device has been brought into a suitable in vivo surrounding, such as a bone cavity. Preferably, said dissolution of the proteins is slow release allowing diffusion of the protein into the tissue which surrounds the device. Thus, the device serves as an in vivo source for osteoinductive proteins which are slowly released and which can be thereby efficiently distributed into the surrounding tissues or have an effect in the immobilized form.
The device may, moreover, comprise additional excipients. These excipients serve to stabilization of the protein, e.g., saccharides, amino acids, polyols or detergents or

maintenance of the pH, e.g., buffer substances. Preferred excipients encompassed by this invention are discussed in detail below.

The term "osteoinductive" refers to the capability of the transformation of mesenchymal stem cells into osteoblasts and chondrocytes. A prerequisite for osteoinduction is a signal which is distributed by the device into the surrounding tissues where the aforementioned osteoblast precursors become activated. Osteoinduction as used herein encompasses the differentiation of mesenchymal cells into the bone precursor cells, the osteblasts. Moreover, osteoinduction also comprises the differentiation of said osteoblasts into osteocytes, the mature cells of the bone. Moreover, also encompassed by osteinduction is the differentiation of mesenchymal cells into chondrocytes. In particular in the long bones, the chondroblasts and the chondrocytes residing in the perichondrium of the bone can also differentiate into osteocytes. Thus, osteoinduction requires differentiation of undifferentiated or less-differentiated cells into osteocytes which are capable of forming the bone. Thus, a prerequisite for osteoinduction is a signal which is distributed by the device into the surrounding tissues where the aforementioned osteocyte precursors usually reside. As has been described above, the osteoinductive proteins used in accordance with the present invention are slowly released from the device after implantation and are distributed efficiently in the surrounding tissues. Moreover, the proteins and polypeptides encompassed by the present invention have osteoinductive properties in vivo. For example, it is well known in the art that the Transforming Growth Factor-ß (TGF-ß) superfamily encompasses members which have osteoinductive properties. Individual members of said TGF-ß superfamily which have particular well osteoinductive properties are listed infra. In conclusion, the osteoinductive proteins of the device of the present invention after having been released from the carrier serving as a osteoinductive signal for the osteocyte precursors of the tissue surrounding the side of implantation of the device.

The term "osteogenic" describes the synthesis of new bone by osteoblasts. In accordance with the present invention, preexisting bone in the surrounding of the side of implantation of the device grows into the device using the structure of the

device as a matrix onto which the osteocytes can adhere.

The term "carrier" encompasses three dimensional matrices, such as the ceramics referred to above. Moreover, as described above, said carrier, preferably, has an enlarged surface due to formation of macro- and micro-pores. The carrier material has a high affinity for osteoinductive proteins but nevertheless allows release of said proteins in vivo. In accordance with the present invention, said carrier is, preferably, a calcium phosphate. The carrier comprised by the device of the invention may be brought into a suitable from for administration of the device in vivo, such as ceramics in form of granules, blocks, cubes, cements and amorphic pastes. In addition, the carrier may be coated onto a metallic surface.

The term "calcium phosphate" encompasses compositions comprising calcium ions, phosphate ions and, optionally, further ions or atoms which are suitable for the carrier of the present invention. The calcium phosphates as used in accordance with the present invention are crystals having a three dimensional structure suitable for the device of the present invention as set forth above. A list of preferred and well known calcium phosphates is given infra.

The term "osteoinductive protein" as set forth above, refers to Transforming Growth Factor-ß (TGF-ß) superfamily members which have osteoinductive properties, such as Growth and Differentiation Factor-5; see infra. These osteoinductive proteins exhibit a high affinity to calcium phosphates. Calcium phosphate can be present e.g. in the form of beta-TCP, α-TCP or hydroxy apatite. Depending on the macro-(100-400 nm) and micropores (< 10 nm), these inorganic minerals absorb aqueous solutions. During this process, proteins such as GDF-5 or BMP-2 are adsorbed tightly onto the surface of the carrier. An important precondition for this process is a sufficient solubility of the proteins in the coating solution

The term "homogeneously coated" means that the surface of the carrier is entirely coated with the said osteoinductive protein, whereby essential identical amounts of protein are present in each and every area of the surface of said carrier. A homogeneously coated carrier in accordance with this invention, preferably, exhibits

a maximum covering with the osteoinductive protein on its surface. Homogenous coating is a prerequisite for efficient release and homogenous distribution and activity of the osteoinductive protein into the tissue surrounding the site of implantation. Moreover, it is to be understood that the osteoinductive proteins are not aggregated and partially or entirely inactivated due to precipitation or micro-precipitation, rather attachment of biologically active, non-aggregated proteins is to be achieved by homogenous coating. Said homogenous coating can be achieved by the method of the present invention and as described in the accompanied Examples. Further, means and methods for controlling homogeneous coating, quantification and characterization of the immobilized protein are described in the accompanied Examples.

Advantageously, it has been found in accordance with the present invention that the above described device of the present invention has improved and reliable osteoinductive and osteoconductive properties in vivo after implantation into a subject, preferably a human. A prerequisite for such a device is a homogenous coating of the carrier with biologically active, non-aggregated osteoinductive protein. It has been found that even aggregation caused by micro-precipitation leads to an inhomogenous coat resulting in at least significantly decreased osteoinductive properties as described for other devices in the prior art, e.g., in WO98/21972.
Moreover, it has been found that undesirable side effects, such as inflammation and toxic reactions of the subject after implantation, can be avoided by the device of the present invention which is free of toxic impurities or infectious contaminants. In particular, the use of protecting proteins (such as e.g. gelatine) as solubility mediator is totally unnecessary for the device of the present invention.

Moreover, the present invention relates to a method for the production of a device having osteoinductive and osteoconductive properties in vivo comprising the steps of:

(a) providing a solution comprising dissolved osteoinductive protein and a buffer which keeps said protein dissolved for a time sufficient to allow homogenous coating of a carrier containing calcium phosphate when brought into contact with said carrier;

(b) contacting the solution of step (a) with a carrier containing calcium

phosphate;

(c) allowing homogenous coating of the surface of said carrier with said dissolved protein; and

(d) drying of the coated carrier obtained in step (c).

The definitions of the terms used to describe the device of the invention apply mutatis mutandis to the aforementioned method and the methods referred to below.

The term "drying" encompasses means for removing liquids, such as excess buffer solution, which are still present after coating of the carrier with the osteoinductive protein. Preferably, drying is achieved by vaccum- or freeze-drying.

The term "buffer which keeps said protein dissolved for a time to allow homogenous coating" refers to a buffer in which the osteoinductive proteins can be efficiently dissolved and which is capable of balancing the increase of pH caused by contacting the buffer solution with the calcium phosphate carrier so that the protein does not immediately precipitate, e.g., due to a pH increase. Said buffer can be composed by the person skilled in the art based on the solubility of the osteoinductive protein which depends on the pH, the ionic strength and the influence of the carrier on said parameters after contacting the carrier with said buffer solution. In accordance with the present invention it has been found that a suitable buffer for the method of the present invention comprises a buffer substance in a low concentration, a weak acid, an alcohol or a saccharide.

An advantage of the present invention is the homogenous coating which is achieved by limitation of the pH increase of the coating solution during the coating process. The described process allows the homogenous distribution and immobilization of the osteoinductive protein onto the said carrier. The efficacy of the coating process is, furthermore, supported by the carrier due to capillary forces resulting from the presence of the numerous macro- and micro-pores which due to their size are capable of soaking the solution into the pores. Moreover, in contrast to other methods described in the art, e.g., in WO98/21972, the osteoinducitve protein or polypeptide is according to the method of the present invention applied by attachment to the carries rather than by precipitation or micro-precipitation. The findings underlying the present invention demonstrate that the aggregation of the proteins can be avoided by the use of suitable additives as described herein. An

important precondition is the knowledge of the solubility of the osteoinductive protein dependent on the pH value, ionic strength and surfaces present. The slowing down of the pH increase caused by the contact of coating solution with the calcium phosphates reacting in an alkaline manner, in particular, plays an important role during the coating. Advantageously, by the method of the present invention, distributing evenly across the inner surface of the carrier material and being able to bind to the surfaces before a pH-induced precipitation of the said protein takes place. It could be demonstrated that the pH increase taking place during the coating of calcium phosphates is decelerated sufficiently by the use of a weak acid, such as acetic acid. Furthermore, the addition of organic combinations such as ethanol or sucrose proves to be additionally advantageous. Furthermore, a low ionic strength are an important precondition for successful coating. Moreover, our tests show that the volume of the coating solution, too, has a considerable effect on the quality of the coating. Finally, the method of the present invention aims to avoid harmful organic solvents, such as acetonitrile, which are routinely used in the methods described in the art. By avoiding said harmful organic solvents, the safety profile and local tolerability of the device of the present invention can be improved.

In a preferred embodiment of the method of the invention said buffer has a buffer concentration of less than 100 mmol/l, less than 50 mmol/l or less than 20 mmol/l.

It follows from the above that more preferably, said buffer contains a weak acid. The term "weak acid" refers to organic or inorganic compounds containing at least one ionogenically bound hydrogen atom. Weak acids are well known in the art and are described in standard text books, such as Römpp, lexicon of chemistry. Preferably, said weak acids which have low dissociation degrees and are described by pK values between 3 and 7, preferred between 4 and 6.

Most preferably, said weak acid is acetic acid or succinic acid.

In another preferred embodiment of the method of the invention said buffer further

comprises saccharides.
The term "saccharides" encompasses mono-, di- and polysaccharides. The structure and composition of mono-, di, and polysaccharides are well known in the art and are described in standard text books, such as Römpp, lexicon of chemistry.

More preferably, said saccharide is a disaccharide. Most preferably, said dissaccharide is sucrose or trehalose.

In a further preferred embodiment of the method of the invention said buffer comprises an alcohol.
Suitable alcohols are well known in the art and are described in standard text books, such as Römpp, lexicon of chemistry.

More preferably, said alcohol is ethanol or mannitol.

In a preferred embodiment of the device or the method of the invention said calcium phosphate is beta tricalcium phosphate, alpha tricalcium phosphate, apatite or a calcium phosphate containing cement.
Said calcium phosphates are particularly well suited as carriers for the device of the present invention. Their in vivo properties have been described in Hotz, 1994, Gao, 1996, and in WO98/21972.

In a further preferred embodiment of the device or the method of the invention said osteoinductive protein is a member of the TGF-ß family.
The TGF-ß family of growth and differentiation factors has been shown to be involved in numerous biological processes comprising bone formation. All members of said family are secreted polypeptides comprising a characteristic domain structure. On the very N-terminus, the TGF-ß family members comprise a signal peptide or secretion leader. This sequence is followed at the C-terminus by the prodomain and by the sequence of the mature polypeptide. The sequence of the mature polypeptide

comprises seven conserved cysteins, six of which are required for the formation of intramolecular disulfide bonds whereas one is required for dimerization of two polypeptides. The biologically active TGF-ß family member is a dimer, preferably composed of two mature polypeptides. The TGF-ß family members are usually secreted as proproteins comprising in addition to the mature sequence the prodomain. The prodomains are extracellularly cleaved off and are not part of the signalling molecule. It has been reported, however, that the prodomain(s) may be required for extracellular stabilization of the mature polypeptides.

In the context of the present invention, the term "TGF-ß family member" or the proteins of said family referred to below encompass all biologically active variants of the said proteins or members and all variants as well as their inactive precursors. Thus, proteins comprising merely the mature sequence as well as proteins comprising the mature protein and the prodomain or the mature protein, the prodomain and the leader sequence are within the scope of the invention as well as biologically active fragments thereof. Whether a fragment of a TGF-ß member has the biological activity can be easily determined by biological assays described, e.g. in: Katagiri T, Yamaguchi A, Ikeda T, Yoshiki S, Wozney JM, Rosen V, Wang EA, Tanka H, Omura S, Suda T, (1990): The non-osteogenic mouse pluripotent cell line, C3H10T1/2, is induced to differentiate into osteoblastic cells by recombinant human bone morphogenetic protein-2. Biochem. Biophys. Res. Commun. 172: 295-299 or Nishitoh H, Ichijo H, Kimura M, Matsumoto T, Makishima F, Yamaguchi A, Yamashita H, Enomoto S, Miyazono K (1996): Identification of type I and type II serine/ threonine kinase receptors for growth/ differentiation factor-5. J. Biol. Chem. 271: 21345-21352.

Preferably, the biological activity according to the invention can be determined by in vivo models as described in the accompanied Examples. Furthermore, encompassed by the present invention are variants of the TGF-ß members which have an amino acid sequences being at least 75%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the amino acid sequences of the members of the TGF-ß family.

An overview of the members of the TGF-ß superfamily is given in: Wozney JM,

Rosen V (1998): Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. Clin Orthop 346: 26-37. The amino acid sequences of the members of the TGF-ß family can be obtained from the well known databases such as Swiss-Prot via the internet (http://www.expasy.ch/sprot/sprot-top.html) Amino acid sequences for BMP2, BMP7 and GDF-5, members of the TGF-β family with a particularly high osteoinductive potential, are also shown in SEQ ID No: 1 to 3, respectively. Amino acid sequences for BMP2, BMP7 and GDF-5, members of the TGF-ß family with a particularly high osteogenic potential, are also shown in SEQ ID No:1 to 3, respectively.

More preferably, said member of the TGF-ß family is a member of the BMP subfamily.

The members of the Bone Morphogenetic Protein (BMP) subfamily have been shown to be involved, inter alia, in the induction and re-modelling of bone tissue. BMPs were originally isolated from bone matrix. These proteins are characterized by their ability to induce new bone formation at ectopic sites. Various in vivo studies demonstrated the promotion of osteogenesis and chondrogenesis of precursor cells by BMPs and raise the possibility that each BMP molecule has distinct role during the skeletal development. More details about the molecular and biological properties of the BMPs are described in:

Wozney JM, Rosen V (1998): Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. Clin Orthop 346: 26-27, Schmitt J, Hwang K, Winn, SR, Hollinger J (1999): Bone morphogenetic proteins: an update on basic biology and clinical relevance. J Orthop Res 17: 269-278 and Lind M (1996): Growth factors: possible new clinical tools. A review. Acta Orthop Scand 67: 407-17.

Most preferably, said member of the BMP family is BMP2 or BMP7.

The amino acid sequence for the preproform of BMP2 is deposited under Swiss-Prot Accession number P12643 and is shown below. Amino acids 1 to 23 correspond to the signal sequence, amino acids 24 to 282 correspond to the propeptide and amino acids 283 to 396 correspond to the mature protein. The amino acid sequence for the preproform of BMP7 is deposited under Swiss-Prot Accession number P18075 or shown in SEQ ID No: 2. Amino acids 1 to 29 correspond to the leader sequence,

amino acids 30 to 292 correspond to the proform and amino acids 293 to 431 correspond to the mature protein. Preferably, BMP-2 or BMP7 refers to the preproform, to the proform or to the mature BMP-2 or BMP-7 peptide, respectively. Moreover also encompassed are fragments of said proteins having essentially the same biological activity, prefrably osteoinductive properties. More sequence information for BMP2 and BMP7 is provided below.

Also more preferably, said member of the TGF-ß family is a GDF.

Growth and Differentiation Factor (GDF) have been also shown to be involved, inter alia, in the induction and re-modelling of bone tissue. Growth Differentiation Factor 5 (GDF-5), also known as cartilage-derived morphogenetic protein 1 (CDMP-1) is a member of subgroup of the BMP family, which also includes other related proteins, preferably, GDF-6 and GDF-7. The mature form of the protein is a 27 kDa homodimer. Various in vivo and in vitro studies demonstrate the role of GDP-5 during the formation of different morphological features in the mammalian skeleton. Mutations of GDF-5 are responsible for skeletal abnormalities including decrease of the length of long bones of limbs, abnormal joint development in the limb and sternum (Storm & Kingsley (1999), Development Biology, 209, 11-27). The amino acid sequence between mouse and human is highly conserved.

Most preferably, said member of the GDF subfamily is GDF-5.

The amino acid sequence for the preproform of GDF-5 is deposited under Swiss-Prot Accession number P 43 0 26 or shown in SEQ ID No: 3. Amino acids 1 to 27 correspond to the leader sequence, amino acids 28 to 381 correspond to the proform and amino acids 382 to 501 correspond to the mature protein. Preferably, GDF-5 refers to the preproform, to the proform or to the mature GDF-5 peptide. Moreover also encompassed are fragments of GDF-5 having essentially the same biological activity, prefrably osteoinductive properties. Most preferably, said fragment comprises amino acids 383 to 501 of the sequence shown in SEQ ID No: 3.

In another preferred embodiment of the device or the method of the invention said device is free of toxic substances.

The term "toxic substances", preferably, encompasses those toxic organic solvents

and additives which are used by the methods described in the art, e.g. actetonitrile. Said substances may cause inflammation and other reactions after implantation of devices containing said substances. Said devices are therapeutically less acceptable due to said undesirable side effects which can not be avoided by the coating methods described in the art. Moreover, the international guidance for the development of therapeutic proteins require that in the manufacturing process harmful and toxic substances should be avoided (for details see: International Conference on Harmonisation (ICH), Topic Q3C; www. emea.eu.int/). However, the device of the present invention or a device which is obtainable by the method of the present invention is, advantageously, free of said toxic substances and, therefore, therapeutically well acceptable and fulfills the requirements of the regulatory authorities.

Moreover, in a further preferred embodiment of the device or the method of the invention said device is free of infectious material.
Besides toxic substances, infectious material comprised by the device may cause severe infections in a subject into which the device has been transplanted. Potentially infectious gelatin derived from bovine or procine bones is, however, used as a protecting protein in many state of the art methods (Lind, 1996).

The invention encompasses a pharmaceutical composition comprising the device of the invention or a device which is obtainable by the method of the invention.
The product of the present invention can be formulated as a pharmaceutical composition or a medical device. The composition of said product may comprise additional compounds like stabilizers, buffer substances and other excipients. The amount of the product of the present invention applied to the patient will be determined by the attending physician and other clinical factors; preferably in accordance with any of the above described methods. As it is well known in the medical arts, the amount applied to a patient depends upon many factors, including the patient's size, body surface area, age, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment.

Thanks to the present invention, it is possible to treat various bone defects including large cavities. In particular, large cavities could not or only under use of autogenous bone material be efficiently treated. However, due to the reliable and efficient osteoinductive and the oseoconductive properties of the device of the present invention or a device which can be obtained by the method of the invention treatment of bone defects which requires extensive bone augmentation or repair has now become possible without a second surgery.

The invention also encompasses the use of the device of the invention or a device which is obtainable by the method of the invention for the preparation of a pharmaceutical composition to be used for bone augmentation.
The definitions of the terms referred to above apply mutatis mutandis to the aforementioned use of the present invention and those described infra.
The term "bone augmentation" refers to the therapeutic formation of bone, which is indicated in order to treat bone defects, cavities in bones, or diseases and disorders accompanied with loss of bone tissue or to prepare the subsequent setting of an implant. The diseases and disorders described in the following are well known in the art and are described in detail in standard medical text books such as Pschyrembel or Stedman.

Preferably, said bone augmentation follows traumatic, malignant or artificial defects.

Another embodiment of the present invention relates to the use of the device of the invention or a device which is obtainable by the method of the invention for the preparation of a pharmaceutical composition for treating bone defects.

More preferably, said bone defects are long bone defects or bone defects following apicoectomy, extirpation of cysts or tumors, tooth extraction, or surgical removal of retained teeth.

The invention also relates to the use of the device of the invention or a device which

is obtainable by the method of the invention for filing of cavities and support guided tissue regeneration in periodontology.

Another embodiment of the present invention relates to the use of the device of the invention or a device which is obtainable by the method of the invention for the preparation of a pharmaceutical composition to be used for sinus floor elevation, augmentation of the atrophied maxillary and mandibulary ridge and stabilization of immediate implants.

Also within the scope of the present invention is a method for treating one or more of the diseases referred to in accordance with the uses of the present invention, wherein said method comprises at least the step of administering the device of the invention or a device which can be obtained by the method of the invention in a pharmaceutically acceptable form to a subject. Preferably, said subject is a human.

Finally, the invention relates to a kit comprising the device of the invention or a device which is obtainable by the method of the invention.
The parts of the kit of the invention can be packaged individually in vials or other appropriate means depending on the respective ingredient or in combination in suitable containers or multicontainer units. Manufacture of the kit follows preferably standard procedures which are known to the person skilled in the art.

The following tables show amino acid sequences for BMP-2, BMP-7 and GDF-5:

Human BMP-2 (Swiss-Prot Prim. Accession Number P12643); SEQ ID No. 1:

Key	From	To	Length
SIGNAL	1	23	23
PROPEP	24	282	259
hBMP2	283	396	114

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        10          20          30          40          50          60
        |           |           |           |           |           |
MVAGTRCLLA LLLPQVLLGG AAGLVPELGR RKFAAASSGR PSSQPSDEVL SEFELRLLSM

        70          80          90          100         110         120
        |           |           |           |           |           |
FGLKQRPTPS RDAVVPPYML DLYRRHSGQP GSPAPDHRLE RAASRANTVR SFHHEESLEE

        130         140         150         160         170         180
        |           |           |           |           |           |
LPETSGKTTR RFFFNLSSIP TEEFITSAEL QVFREQMQDA LGNNSSFHHR INIYEIIKPA

        190         200         210         220         230         240
        |           |           |           |           |           |
TANSKFPVTR LLDTRLVNQN ASRWESFDVT PAVMRWTAQG HANHGFVVEV AHLEEKQGVS

        250         260         270         280         290         300
        |           |           |           |           |           |
KRHVRISRSL HQDEHSWSQI RPLLVTFGHD GKGHPLHKRE KRQAKHKQRK RLKSSCKRHP

        310         320         330         340         350         360
        |           |           |           |           |           |
LYVDFSDVGW NDWIVAPPGY HAFYCHGECP FPLADHLNST NHAIVQTLVN SVNSKIPKAC

        370         380         390
        |           |           |
CVPTELSAIS MLYLDENEKV VLKNYQDMVV EGCGCR
```

References

[1] SEQUENCE FROM NUCLEIC ACID.
MEDLINE=89072730; PubMed=3201241;
Wozney J.M., Rosen V., Celeste A.J., Mitsock L.M., Whitters M.J., Kriz R.W., Hewick R.M., Wang E.A.;
"Novel regulators of bone formation: molecular clones and activities.";
Science 242:1528-1534(1988).

[2] X-RAY CRYSTALLOGRAPHY (2.7 ANGSTROMS) OF 292-396.
MEDLINE=99175323; PubMed=10074410;
Scheufler C., Sebald W., Huelsmeyer M.;
"Crystal structure of human bone morphogenetic protein-2 at 2.7 A resolution.";
J. Mol. Biol. 287:103-115(1999).

Human BMP-7(Swiss-Prot Prim. Accession. Number: P18075); SEQ ID No. 2:

Key	From	To	Length
SIGNAL	1	29	29

PROPEP	30	292	263
hBMP-7	293	431	139

```
         10          20          30          40          50          60
         |           |           |           |           |           |
MHVRSLRAAA PHSFVALWAP LFLLRSALAD FSLDNEVHSS FIHRRLRSQE RREMQREILS

         70          80          90         100         110         120
         |           |           |           |           |           |
ILGLPHRPRP HLQGKHNSAP MFMLDLYNAM AVEEGGGPGG QGFSYPYKAV FSTQGPPLAS

        130         140         150         160         170         180
         |           |           |           |           |           |
LQDSHFLTDA DMVMSFVNLV EHDKEFFHPR YHHREFRFDL SKIPEGEAVT AAEFRIYKDY

        190         200         210         220         230         240
         |           |           |           |           |           |
IRERFDNETF RISVYQVLQE HLGRESDLFL LDSRTLWASE EGWLVFDITA TSNHWVVNPR

        250         260         270         280         290         300
         |           |           |           |           |           |
HNLGLQLSVE TLDGQSINPK LAGLIGRHGP QNKQPFMVAF FKATEVHFRS IRSTGSKQRS

        310         320         330         340         350         360
         |           |           |           |           |           |
QNRSKTPKNQ EALRMANVAE NSSSDQRQAC KKHELYVSFR DLGWQDWIIA PEGYAAYYCE


        370         380         390         400         410         420
         |           |           |           |           |           |
GECAFPLNSY MNATNHAIVQ TLVHFINPET VPKPCCAPTQ LNAISVLYFD DSSNVILKKY

        430
         |
RNMVVRACGC H
```

References

[1] SEQUENCE FROM NUCLEIC ACID, AND PARTIAL SEQUENCE.
TISSUE=Placenta;
MEDLINE=90291971; PubMed=2357959;
Oezkaynak E., Rueger D.C., Drier E.A., Corbett C., Ridge R.J., Sampath T.K., Oppermann H.;
"OP-1 cDNA encodes an osteogenic protein in the TGF-beta family.";
EMBO J. 9:2085-2093(1990).

[2] SEQUENCE FROM NUCLEIC ACID.
MEDLINE=91088608; PubMed=2263636;
Celeste A.J., Iannazzi J.A., Taylor R.C., Hewick R.M., Rosen V., Wang E.A., Wozney J.M.;
"Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone.";
Proc. Natl. Acad. Sci. U.S.A. 87:9843-9847(1990).

[3] X-RAY CRYSTALLOGRAPHY (2.8 ANGSTROMS) OF 293-431.
MEDLINE=96149402; PubMed=8570652;
Griffith D.L., Keck P.C., Sampath T.K., Rueger D.C., Carlson W.D.;
"Three-dimensional structure of recombinant human osteogenic protein 1: structural paradigm for the transforming growth factor beta superfamily.";
Proc. Natl. Acad. Sci. U.S.A. 93:878-883(1996).

Human GDF-5 (Swiss-Prot Prim. Accession Number: P 43026); SEQ ID No. 3:

Key	From	To	Length
SIGNAL	1	27	27
PROPEP	28	381	354
hGDF-5	382	501	120

```
         10         20         30         40         50         60
          |          |          |          |          |          |
MRLPKLLTFL LWYLAWLDLE FICTVLGAPD LGQRPQGSRP GLAKAEAKER PPLARNVFRP

         70         80         90        100        110        120
          |          |          |          |          |          |
GGHSYGGGAT NANARAKGGT GQTGGLTQPK KDEPKKLPPR PGGPEPKPGH PPQTRQATAR

        130        140        150        160        170        180
          |          |          |          |          |          |
TVTPKGQLPG GKAPPKAGSV PSSFLLKKAR EPGPPREPKE PFRPPPITPH EYMLSLYRTL

        190        200        210        220        230        240
          |          |          |          |          |          |
SDADRKGGNS SVKLEAGLAN TITSFIDKGQ DDRGPVVRKQ RYVFDISALE KDGLLGAELR

        250        260        270        280        290        300
          |          |          |          |          |          |
ILRKKPSDTA KPAVPRSRRA AQLKLSSCPS GRQPAALLDV RSVPGLDGSG WEVFDIWKLF

        310        320        330        340        350        360
          |          |          |          |          |          |
RNFKNSAQLC LELEAWERGR TVDLRGLGFD RAARQVHEKA LFLVFGRTKK RDLFFNEIKA

        370        380        390        400        410        420
          |          |          |          |          |          |
RSGQDDKTVY EYLFSQRRKR RAPLATRQGK RPSKNLKARC SRKALHVNFK DMGWDDWIIA

        430        440        450        460        470        480
          |          |          |          |          |          |
PLEYEAFHCE GLCEFPLRSH LEPTNHAVIQ TLMNSMDPES TPPTCCVPTR LSPISILFID

        490        500
          |          |
SANNVVYKQY EDMVVESCGC R
```

References

[1] SEQUENCE FROM NUCLEIC ACID.
TISSUE=Placenta;
MEDLINE=95071375; PubMed=7980526;
Hoetten G., Neidhardt H., Jacobowsky B., Pohl J.;
"Cloning and expression of recombinant human growth/differentiation factor 5.";
Biochem. Biophys. Res. Commun. 204:646-652(1994).

[2] SEQUENCE FROM NUCLEIC ACID.
TISSUE=Articular cartilage;
MEDLINE=95050604; PubMed=7961761;
Chang S., Hoang B., Thomas J.T., Vukicevic S., Luyten F.P., Ryba N.J.P., Kozak C.A., Reddi A.H., Moos M.;
"Cartilage-derived morphogenetic proteins. New members of the transforming growth factor-beta superfamily predominantly expressed in long bones during human embryonic development.";
J. Biol. Chem. 269:28227-28234(1994).

The figures show:

Figure 1: Solubility of GDF-5 in 5 mM acetic acid, 5 mM H3PO4/NaOH, 150 mM NaCl at different pH values.

Figure 2: Solubility of GDF-5 in 20 mM Arginin/acetic acid at different pH values.

Figure 3: Solubility of GDF-5 in two buffers having different ionic strength and pH values (HAc = acetic acid).

Figure 4: Increase of the pH during coating in the presence of 10 mmol/l HCl.

Figure 5: Increase of the pH in the presence of 75 % acetonitrile.

Figure 6: Dependency of the pH of the coating solutions having different concentrations of acetic acid during coating.

Figure 7: Homogeneity of the distribution of GDF-5 on beta-TCP (100 µg GDF-5 on 100 mg beta-TCP) achieved by coating in the presence of 10 mmol/l acetic acid with (right) and without sucrose (left).

Figure 8: Homogeneity of the distribution of GDF-5 on beta-TCP (100 µg GDF-5 on 100 mg beta-TCP) achieved by coating in the presence of 60 % ethanol.

Figure 9: Homogeneity of the distribution of GDF-5 on beta-TCP (100 µg GDF-5 on 100 mg beta-TCP) achieved by coating in the presence of 20 mmol/l glycin/NaOH, pH 10.

Figure 10: Histomorphometrical analysis of an implant coated in the presence of 20 mmol/l glycin/NaOH, pH10.

Figure 11: Histomorphometrical analysis of an implant coated in the presence of 60 % ethanol.

Figure 12: Homogeneity of the distribution of rhBMP-2 on beta-TCP.

Figure 13: Homogeneity of the distribution of GDF-5 on beta-TCP achieved by coating in the presence of sucrose (left) and trehalose (right).

Figure 14: Homogeneity of the distribution of GDF-5 on beta-TCP achieved by coating in the presence of ethanol (left) and mannitol (right).

The invention will now be described by reference to the following biological Examples which are merely illustrative and are not constructed as a limitation of the scope of the present invention.

Example1: Quantification of GDF-5 in solution by RP-HPLC

The GDF-5 content was determined by reversed phase (RP-) HPLC-analysis. Aliquots of the sample were analysed using a Poros C8-18 column (R2/10, 2.1* 30 mm, Applied Biosystems). 0.1% formic acid in 21% acetonitrile (solvent A) and 0.1 % formic acid in 84 % acetonitrile (solvent B) were used as solvents at a flow rate of 0.4 ml/min. The elution profile was recorded by measuring the absorbance at 220 nm. The amounts of GDF-5 were calculated form the peak area at 220 nm using a standard curve.

Example 2: Extraction and quantification of the immobilised protein

Method A:
Coated beta-TCP (40 mg) were suspended in 700 µl solution matrix (1.22 mol/l citric acid, 1.22 mol/l HCl, 8 mol/l urea) and incubated for 60 min at 4°C. After centrifugation (13200* g, 2 min) 50 µl of the supernatant was analysed by RP_HPLC

(see example 1). The standard curve was taken with various amounts of GDF-5 in the respective matrix solution.

Method B:
Coated beta-TCP (40 mg) were suspended in 700 µl solution matrix (10 mmol/l Tris/HCl, pH 7.4, 8 mol/l urea, 100 mmol/l EDTA) an incubated for 60 min at 4°C and centrifuged (5 min at 13,500 * g). Subsequently, the supernatant is quantified as described in method A or analysed further.

Example 3: Solubility of rhGDF-5 at different pH values

GDF-5 is adjusted to a concentration of 4 mg/ml 10 mmol/l HCl. Aliquots (50 µl) of the stock solution are diluted 1:100 with 5 mmol/l acetic acid, 5 mmol/l H_3PO_4/NaOH, 150 mmol/l NaCl with different pH values each. The samples were incubated for 15 min and centrifuged (2 min at 13,200 * g). The pH value and the protein content in the supernatant were determined. In a second test, the stock solution was diluted with 20 mmol/l arginine/HOAc with different pH values each. The data in Figures 1 and 2 show that GDF-5 in buffers with low ionic strength (≤ 20 mmol/l) is soluble only in an acidic (pH ≤ 5) or in an alkaline (> pH 10) solution. In the pH range of between 6.0 and 9.5, however, the solubility is < 5 µg/ml.

Example 4: Solubility of GDF-5 in two buffers having different ionic strength at different pH values

GDF-5 is adjusted to a concentration of 4 mg/ml 10 mmol/l HCl. Aliquots (50 µl) of the stock solution are diluted 1:100 with 10 mmol/l acetic acid/NaOH and with 5 mmol/l acetic acid, 5 mmol/l H_3PO_4/NaOH, 150 mmol/l NaCl with different pH values each. The samples were incubated for 15 min and centrifuged (2 min at 13,200 * g). The pH value and the protein content in the supernatant are determined. The data in Figure 3 show that with all the pH values measured, the solubility of GDF-5 in a

buffer with higher ionic strength which corresponds to the physiological condition is significantly lower than in the buffer with low ionic strength (about 10 mmol/l).

Example 5: Solubility of GDF-5 in different solvents

Freeze-dried GDF-5 was dissolved in pure acetonitrile, incubated for 15 min at room temperature and centrifuged (13,200* g, 2 min). No GDF-5 was detectable in the supernatant.

Freeze-dried GDF-5 (50 µg) was dissolved with 50 µl 75% acetonitrile, incubated for 15 min at room temperature and centrifuged (13,200 * g, 2 min). In the supernatant, the pH was measured and the content of GDF-5 was determined. 100% of the GDF-5 used were detected, the pH value of the solution was 3.0. Subsequently, the pH value was adjusted to pH 7.4 by adding NaOH, incubated for 15 min at room temperature again and centrifuged. Only 3 µg/ml corresponding to a solubility of 60 µg/ml, were detected.

The data show that GDF-5 is not soluble in pure acetonitrile but that it is soluble in acidic aqueous solutions containing acetonitrile. According to the results found in the aqueous systems, the solubility decreases in acetonitrile-water-mixtures with increasing pH.

Example 6: Change of the pH value of the GDF-5 solution during the coating of beta-TCP

In a reaction vessel, 200 mg beta-TCP are mixed with 200 µl of a coating solution containing GDF-5 (1 mg/ml; produced from a lyophilisate produced from a HCl solution). The pH value of the suspension is observed for 30 min. The results illustrated in Figures 4-5 show that after about 2 min, the pH of the suspension

reaches the range of pH > 6.5, which is critical for the solubility of GDF-5, when unbuffered coating solutions such as e.g. 10 mmol/l HCl or 75% acetonitrile are used (the acidic initial pH in 75% acetonitrile results from the remaining amounts of HCl present). Due to the insufficient solubility, precipitation of the protein and aggregation formation takes place which can be detected by means of Coomassie staining (see Example 7).

The use of an acetate-buffered coating solution (Figure 6) causes a reduction of the pH increase during coating. While the pH increases to up to 8 in the unbuffered solutions, the pH of the acetate (40-80 mmol/l)-buffered coating solution reaches its maximum at pH 5.4. Thus, a sufficient solubility during the coating process is guaranteed. The GDF-5 used can spread evenly and bind to the carrier without precipitation taking place (see Example 7).

A delay of the pH increase is achieved also by using 60% ethanol. The delay is sufficient to achieve an even distribution of GDF-5 across the carrier (see Example 7).

Example 7: Detection of the homogeneity of the coating

The adsorbed protein is made visible by staining with Coomassie Brilliant Blue on the carrier. The distribution of the blue colour correlates with the distribution of the respective protein on the beta-TCP carrier.

3-4 coated granules are incubated with 200 µl staining solution (60% PBS, 40% methanol, 0.4% Coomassie Brilliant Blue R250) in a cavity of a 96-well plate and incubated for 30 min at room temperature. An uncoated carrier is treated in the same way as control. The surplus staining agent is removed by washing with 60% PBS, 40% methanol until the uncoated carrier used as control is completely destained. The stained carrier is dried at 40°C and documented photographically.

Example 8: Coating of granules (I)

200 mg β-TCP (0.5 – 1.0 mm granule size) are placed in a dry form in a 2R-glass. The stock solution of rhGDF-5 (4 mg/ml in 10 mM HCl) is diluted to 1 µg/ml with the means of the corresponding coating buffer. 200 µl of the GDF-5 solution obtained in that manner are pipetted on the beta-TCP and absorbed. The damp granulate is incubated for 1 hour at 25°C and then vacuum-dried.

Example 9: Coating of granules (II)

200 mg β-TCP (0.5 – 1.0 mm granule size) are placed in a dry form in a 2R-glass. The stock solution of rhGDF-5 (4 mg/ml in 10 mM HCl) is diluted to 1 µg/ml with the means of the corresponding coating buffer. 200 µl of the GDF-5 solution obtained in that manner are pipetted on the beta-TCP and absorbed. The damp granulate is incubated for 1 hour at 25°C and then lyophilised.

Example 10: Coating of blocks

A beta-TCP block having a mass of 360 mg is put into a suitable reaction vessel (Eppendorf), mixed with 500 µl of the coating solution, incubated for one hour and then vacuum- or freeze-dried.

Example 11: Comparison of different coating methods

Due to the use of acetate-buffered coating solutions, the precipitation formation could be reduced significantly. Another improvement was achieved by adding sucrose. The quality of the coating with and without sucrose is illustrated in Figure 7. While individual precipitates can still be recognised as dark-blue spots without sucrose, the coating in the presence of sucrose results in a spot-free coating.

The significance of the homogeneity of the coating becomes clear comparing two preparations which were produced during the research using coating solutions in

60% ethanol and 20 mmol/l glycine/NaOH, pH 10, respectively. While a homogenous distribution was achieved in the presence of 60% ethanol (Figure 8), a significant precipitation formation takes place on the carrier surface in the presence of glycine (Figure 9). Both carriers were compared in a rat calvarial defect model (see below).

Example 12: Full-thickness Calvarial Defect Model in Rats

Beta-TCP coated with rhGDF-5 (50 µg/25 mg of beta-TCP) was manufactured using different coating buffers (20 mmol/l glycin/NaOH, pH 10 (C1) and 60% ethanol (C2)). Rats were anaesthetized by intramuscular injection of Tiletamine-Zolazepam (ZOLETIL® VIRBAC, CARROS, France, 50 mg/kg, IM). The dorsal part of the cranium was clipped free of fur. Then, the skin was scrubbed with a germicidal soap (VETEDINE®, VETOQUINOL, LURE, France). The surgical site was scrabbed with an antiseptic such as Povidone iodine (VETEDINE® solution, VETOQUINOL, LURE, France). A 20 mm long incision in the scalp along the sagittal suture was realized and the skin, musculature and periosteum were reflected, exposing the parietal bones. A 6 mm trephined bur (COVELY, GENAY, France) was used to create the defect in the dorsal part of the parietal bone lateral to the sagittal suture under constant irrigation with sterile physiologic solution (AGUETTANT, LYON, France). Two identical defects were created per animal. Care was taken to prevent damage to the dura-mater and to prevent puncture of the superior sagittal sinus. After the implants were applied the periosteum and muscles were sutured in place and the scalp was sutured (polypropylene thread, Prolène®, ETHNOR, ISSY LES MOULINEAUX, France).

After a follow-up of 6 weeks, the animals were anesthetized by intramuscular injection of ZOLETIL® (50 mg/kg) then euthanatized by lethal dosis injection of DOLETHALND (Pentobarbital sodique, VETOQUINOL, LURE, France).

The explants were sampled and fixed in 10 % buffered formalin solution. Afterwards samples were dehydrated in alcohol solutions of increased concentrations and embedded in PMMA (polymethylmetacrylate, Merck KGaA, Darmstadt, Germany). A

section of 20 μm thickness was obtained by a microcutting and grinding technique adapted from Donath (Donath K. Breuner G., A method for the study of undecalcified bone and teeth with attached soft tissues. J. Oral. Pathol. 11, 318-326, 1982). A section was stained with modified Paragon for qualitative and semi-quantitative light microscopy analysis.
Histological sections were observed using a Polyvar microscope (REICHERT) fitted with a x4, x10, x25 and x40 objective.

Large amounts of bone marrow and osteoblastic cells were observed in the C2 treated site. In contrast, bone formation was poor with C1. Degradation of the implant material was also increased with C2 as compared to C1. For the results of the histomorphometrical analyses of the implant materials see Table 1 and Figures 10 and 11.

Table 1

Sample	Bone tissue %	Implant %	Lacunae tissue %	Fibrous tissue %
C1	8,9	39,2	2,1	49,8
C2	43,4	18,5	21,4	16,7

Example 13: Coating of beta-TCP with BMP-2

200 mg beta TCP (0.5 – 1.0 mm granule size) are filled in a 2R-glass. The stock solution of BMP-2 is diluted to 1 mg/ml with the corresponding coating buffer (10 mmol/l acetic acid, 10 % sucrose). 200 µl of the coating solution are incubated with the beta-TCP (1 hr, 4 °C) and freeze dried. The distribution of BMP-2 on the coated carrier was shown by Commassie staining (Figure 12).

Example 14: Comparison of the coating process in the presence of sucrose and trehalose

200 mg beta-TCP (0.5 – 1.0 mm granule size) are filled in a 2R-glass. The stock solution of GDF-5 (4 mg/ml in 10 mmol/l HCl) is diluted to 1 mg/ml with the corresponding coating buffer. The two variants of the coating buffer are containing 10 % sucrose and 10 % trehalose, respectively. 200 µl of the coating solution are incubated with the beta-TCP (1 hr, 4 °C) and freeze dried. The distribution of GDF-5 on the coated carrier was shown by Commassie staining (Figure 13).

Example 15: Comparison of the coating in the presence of ethanol and mannitol

200 mg beta-TCP (0.5 – 1.0 mm granule size) are filled in a 2R-glass. The stock solution of GDF-5 (4 mg/ml in 10 mmol/l HCl) is diluted to 1 mg/ml with the corresponding coating buffer. The two variants of the coating buffer contain 60 % ethanol and 10 % mannitol, 10 mmol/l acetic acid, respectively. 200 µl of the coating solution are incubated with the beta-TCP (1 hr, 4 °C) and freeze dried. The distribution of GDF-5 on the coated carrier was shown by Commassie staining (Figure 14).

References:

Celeste A.J., et al. (1990) "Identification of transforming growth factor ..."; Proc. Natl. Acad. Sci. U.S.A. 87:9843-9847.

Chang, S. et al. (1994); "Cartilage-derived morphogenetic proteins..."; J. Biol. Chem. 269: 28227-28234.

EMEA, ICH Topic Q 3 C, Impurities: Residual Solvents

Friess, W. et al. (1998); Pharm. Dev. Technol. 4, 387 – 396.

Gao, T et al. (1996); Int. Orthopaedics 20, 321 - 325.

Gombotz, W et al. (1996) in Formulation, characterization and stability of protein drugs, Plenum Press, New York, USA, pp 219 – 245.

Griffith, D.L. et al. (1996); "Three-dimensional structure of recombinant human..."; Proc. Nati. Acad. Sci. U.S.A. 93: 878-883.

Hoetten, G. et al. (1994); "Coning and expression of recombinant human growth/differentiation factor 5."; Biochem. Biophys. Res. Commun. 204: 646-652.

Hotz, G et al. (1994); Int. J. Oral Maxillofac. Surg. 23, 413 – 417.

Katagiri, T. et al. (1990); Biochem. Biophys. Res. Commun. 172: 295-299.

Lind, M et al. (1996); J. Orthopaedic Res. 14, 343 – 350

Lind, M. (1996); Acta. Orthop. Scand. 67: 407-17.

Nishitoh, H. et al (1996); J Biol. Chem. 271: 21345-21352.

Oezkayanak, E. et al. (1990); "OP-1 cDNA encodes an osteogenic protein in the TGF-beta family"; EMBO J. 9: 2085-2093

Scheufler, C. et al. (1990); "Crystal structure of human bone morphogenetic protein-2 at 2.7 A resolution".

Schmitt, J. et al. (1999); J. Orthop. Res. 17: 269-278.

Shore, E.M. et al. (1997); "Human bone morphogenetic protein-2 (BMP-2) genomic DNA sequence".

Storm & Kingsley (1999); Development Biology, 209, 11-27.

Terheyden, H et al. (1997); Mund Kiefer Gesichtschir. 1, 272 – 275.

Wang, E.A. et al. (1990); "Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone."; Proc. Natl. Acad. Sci. U.S.A. 87: 9843-9847.

Wozney, J.M. et al. (1998); Clin. Orthop. 346: 26-37.

Wozney, J.M. et al. (1988); Science 242: 1528-1534.

Claims

1. A device having osteoinductive and osteoconductive properties in vivo comprising a carrier containing calcium phosphate and an osteoinductive protein, wherein said carrier is homogeneously coated with said protein.

2. A method for the production of a device having osteoinductive and osteoconductive properties in vivo comprising the steps of:
   (a) providing a solution comprising dissolved osteoinductive protein and a buffer which keeps said protein dissolved for a time sufficient to allow homogenous coating of a carrier containing calcium phosphate when brought into contact with said carrier;
   (b) contacting the solution of step (a) with a carrier containing calcium phosphate;
   (c) allowing homogenous coating of the surface of said carrier with said dissolved protein; and
   (d) drying of the coated carrier obtained in step (c).

3. The method of claim 2, wherein said buffer has a buffer concentration of less than 100 mmol/l, less than 50 mmol/l or less than 20 mmol/l.

4. The method of claim 3, wherein said buffer contains a weak acid.

5. The method of claim 4, wherein said weak acid is acetic acid or succinic acid.

6. The method of any one of claims 2 to 5, wherein said buffer further comprises saccharides.

7. The method of claim 6, wherein said saccharide is a disaccharide.

8. The method of claim 7, wherein said dissaccharide is sucrose or trehalose.

9. The method of any one of claims 2 to 8, wherein said buffer comprises an

alcohol.

10. The method of claim 9, wherein said alcohol is ethanol or mannitol.

11. The device of claim 1 or the method of any one of claims 2 to 10, wherein said calcium phosphate is beta tricalcium phosphate, alpha tricalcium phosphate, apatide or a calcium phosphate containing cement.

12. The device of claim 1, the method of any one of claims 2 to 10 or the device or method of claim 11, wherein said osteoinductive protein is a member of the TGF-ß family.

13. The device or method of claim 12, wherein said member of the TGF-ß family is a member of the BMP subfamily.

14. The device or method of claim 13, wherein said member of the BMP family is BMP2 or BMP7.

15. The device or method of claim 12, wherein said member of the TGF-ß family is a protein of the group of GDF-5, GDF-6 and GDF-7.

16. The device or method of claim 15, wherein said GDF is GDF-5.

17. The device of claim 1, the method of any one of claims 2 to 10 or the device or method of any one of claims 11 to 16, wherein said device is free of toxic substances.

18. The device of claim 1, the method of any one of claims 2 to 10 or the device or method of any one of claims 11 to 17, wherein said device is free of infectious material.

19. A pharmaceutical composition comprising the device of any one of claims 1, or 11 to 18 or which is obtainable by the method of any one of claims 2 to 18.

20. Use of the device of any one of claims 1, or 11 to 18 or which is obtainable by the method of any one of claims 2 to 18 for the preparation of a pharmaceutical composition to be used for bone augmentation.

21. The use of claim 20, wherein said bone augmentation follows traumatic, malignant or artificial defects or is a prerequisite for the subsequent setting of an implant.

22. Use of the device of any one of claims 1, or 11 to 18 or which is obtainable by the method of any one of claims 2 to 18 for the preparation of a pharmaceutical composition for treating bone defects.

23. The use of claim 22, wherein said bone defects are long bone defects, defects in the maxillofacial area or bone defects following apicoectomy, extirpation of cysts or tumors, tooth extraction, or surgical removal of retained teeth.

24. Use of the device of any one of claims 1, or 11 to 18 or which is obtainable by the method of any one of claims 2 to 18 for the preparation of a pharmaceutical composition for treating degenerative and traumatic disc disease.

25. Use of the device of any one of claims 1, or 11 to 18 or which is obtainable by the method of any one of claims 2 to 18 for the preparation of a pharmaceutical composition for treating bone dehiscence.

26. Use of the device of any one of claims 1, or 11 to 18 or which is obtainable by the method of any one of claims 2 to 18 for the preparation of a pharmaceutical composition to be used for sinus floor elevation or augmentation of the atrophied maxillary or mandibular ridge.

27. A kit comprising the device of any one of claims 1, or 11 to 18 or which is obtainable by the method of any one of claims 2 to 18.

AMENDED CLAIMS

[received by the International Bureau on 05 March 2003 (05.03.03);
original claims 1-27 replaced by amended claims 1-26]

1. A device having osteoinductive and osteoconductive properties in vivo comprising a carrier containing calcium phosphate and an osteoinductive protein, wherein said carrier is homogeneously coated with said protein.

2. A method for the production of a device having osteoinductive and osteoconductive properties in vivo comprising the steps of:
   (a) providing a solution comprising dissolved osteoinductive protein and a buffer containing a weak acid having a pK value between 3 and 7, preferably between 4 and 6, said buffer keeping said protein dissolved for a time sufficient to allow homogenous coating of a carrier containing calcium phosphate when brought into contact with said carrier and said buffer being capable of balancing the increase of pH caused by contacting the buffer solution with the calcium phosphate carrier so that the protein does not immediately precipitate because of said pH increase;
   (b) contacting the solution of step (a) with a carrier containing calcium phosphate;
   (c) allowing homogenous coating of the surface of said carrier with said dissolved protein; and
   (d) drying of the coated carrier obtained in step (c).

3. The method of claim 2, wherein said buffer has a buffer concentration of less than 100 mmol/l, less than 50 mmol/l or less than 20 mmol/l.

4. The method of claim 3, wherein said weak acid is acetic acid or succinic acid.

5. The method of any one of claims 2 to 4, wherein said buffer further comprises saccharides.

6. The method of claim 5, wherein said saccharide is a disaccharide.

7. The method of claim 6, wherein said dissaccharide is sucrose or trehalose.

8. The method of any one of claims 2 to 7, wherein said buffer comprises an alcohol.

9. The method of claim 8, wherein said alcohol is ethanol or mannitol.

10. The device of claim 1 or the method of any one of claims 2 to 9, wherein said calcium phosphate is beta tricalcium phosphate, alpha tricalcium phosphate, apatide or a calcium phosphate containing cement.

11. The device of claim 1, the method of any one of claims 2 to 9 or the device or method of claim 10, wherein said osteoinductive protein is a member of the TGF-ß family.

12. The device or method of claim 11, wherein said member of the TGF-ß family is a member of the BMP subfamily.

13. The device or method of claim 12, wherein said member of the BMP family is BMP2 or BMP7.

14. The device or method of claim 11, wherein said member of the TGF-ß family is a protein of the group of GDF-5, GDF-6 and GDF-7.

15. The device or method of claim 14, wherein said GDF is GDF-5.

16. The device of claim 1, the method of any one of claims 2 to 9 or the device or method of any one of claims 10 to 15, wherein said device is free of toxic substances.

17. The device of claim 1, the method of any one of claims 2 to 9 or the device or method of any one of claims 10 to 16, wherein said device is free of infectious material.

18. A pharmaceutical composition comprising the device of any one of claims 1, or 10 to 17 or which is obtainable by the method of any one of claims 2 to 17.

19. Use of the device of any one of claims 1, or 10 to 17 or which is obtainable by the method of any one of claims 2 to 17 for the preparation of a pharmaceutical composition to be used for bone augmentation.

20. The use of claim 19, wherein said bone augmentation follows traumatic, malignant or artificial defects or is a prerequisite for the subsequent setting of an implant.

21. Use of the device of any one of claims 1, or 10 to 17 or which is obtainable by the method of any one of claims 2 to 17 for the preparation of a pharmaceutical composition for treating bone defects.

22. The use of claim 21, wherein said bone defects are long bone defects, defects in the maxillofacial area or bone defects following apicoectomy, extirpation of cysts or tumors, tooth extraction, or surgical removal of retained teeth.

23. Use of the device of any one of claims 1, or 10 to 17 or which is obtainable by the method of any one of claims 2 to 17 for the preparation of a pharmaceutical composition for treating degenerative and traumatic disc

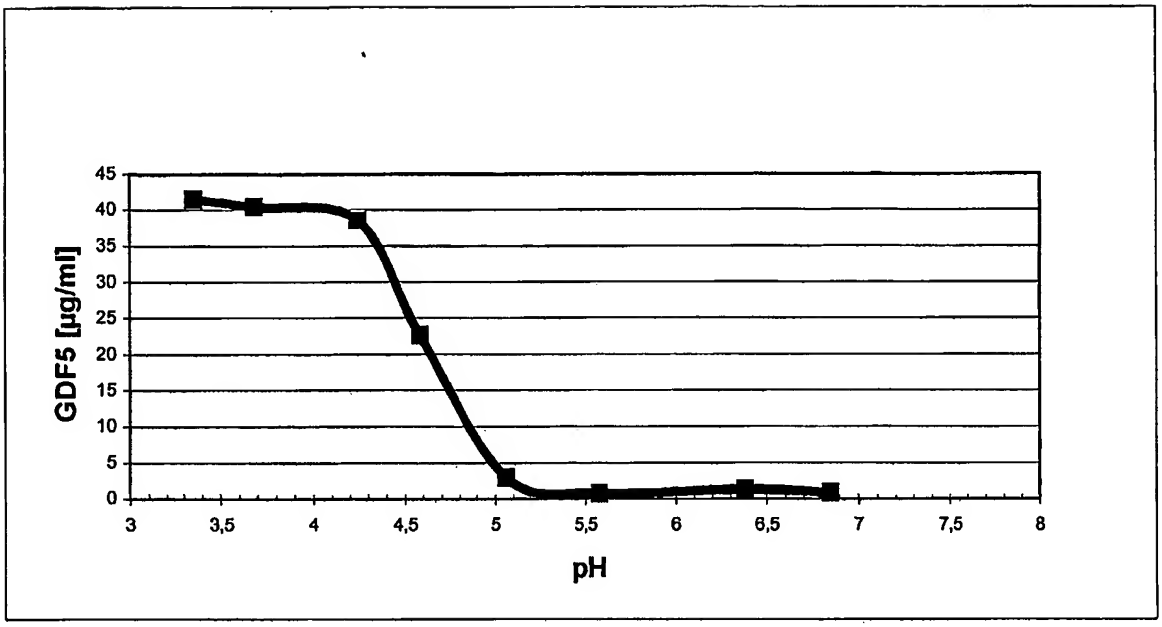
disease.

24. Use of the device of any one of claims 1, or 10 to 17 or which is obtainable by the method of any one of claims 2 to 17 for the preparation of a pharmaceutical composition for treating bone dehiscence.

25. Use of the device of any one of claims 1, or 10 to 17 or which is obtainable by the method of any one of claims 2 to 17 for the preparation of a pharmaceutical composition to be used for sinus floor elevation or augmentation of the atrophied maxillary or mandibular ridge.

26. A kit comprising the device of any one of claims 1, or 10 to 17 or which is obtainable by the method of any one of claims 2 to 17.

Figure 1



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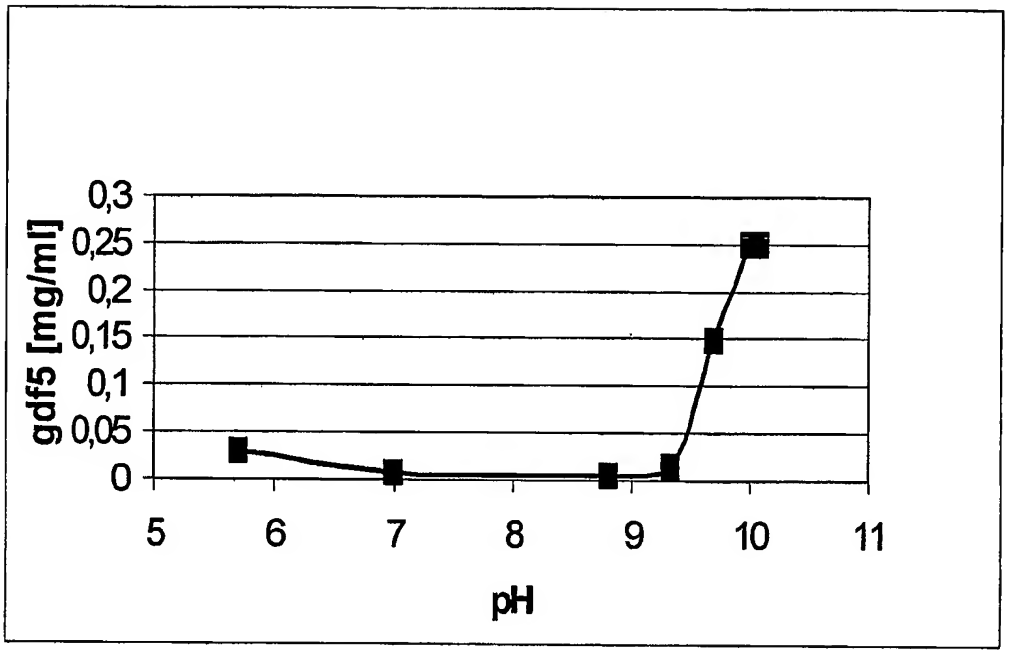

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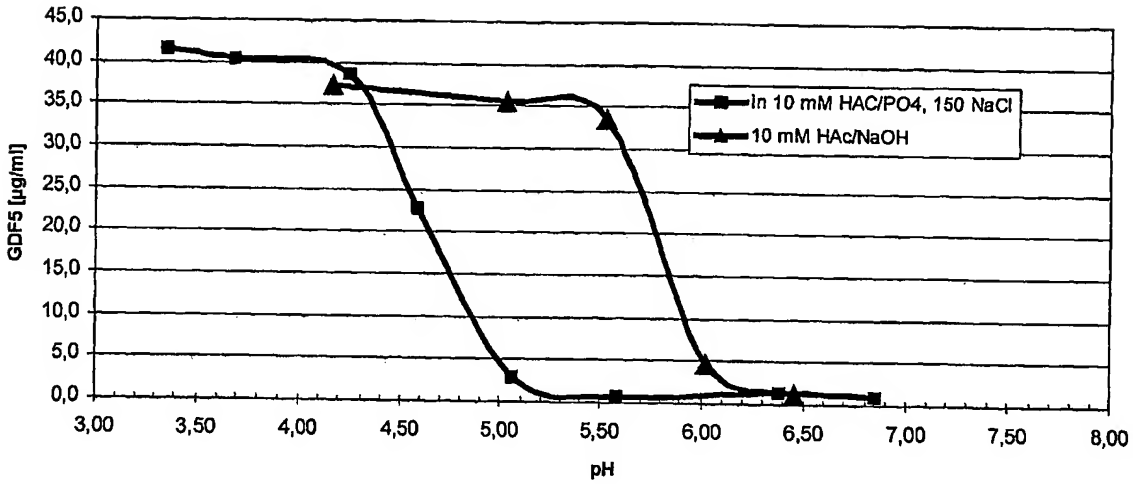
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pH
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10 mM HAc/NaOH

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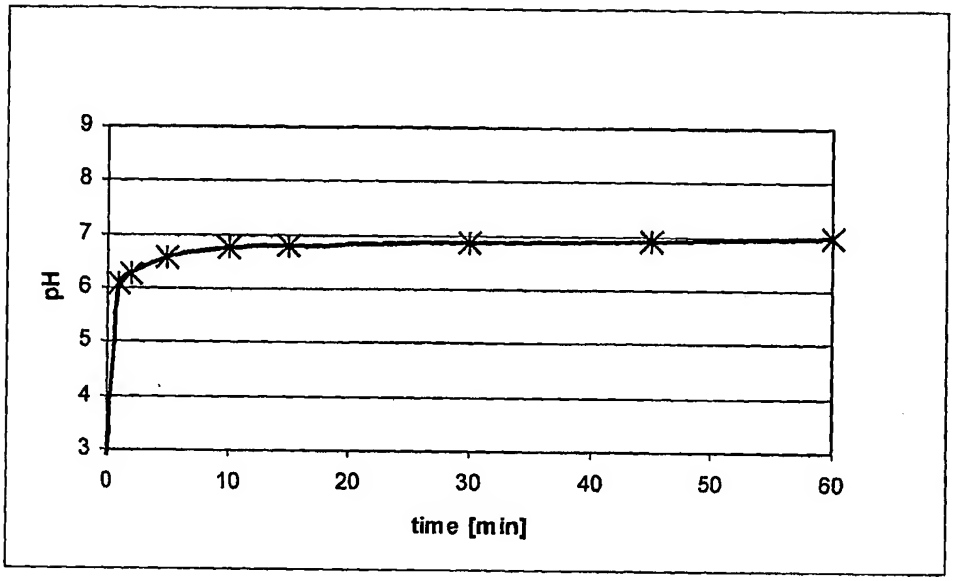

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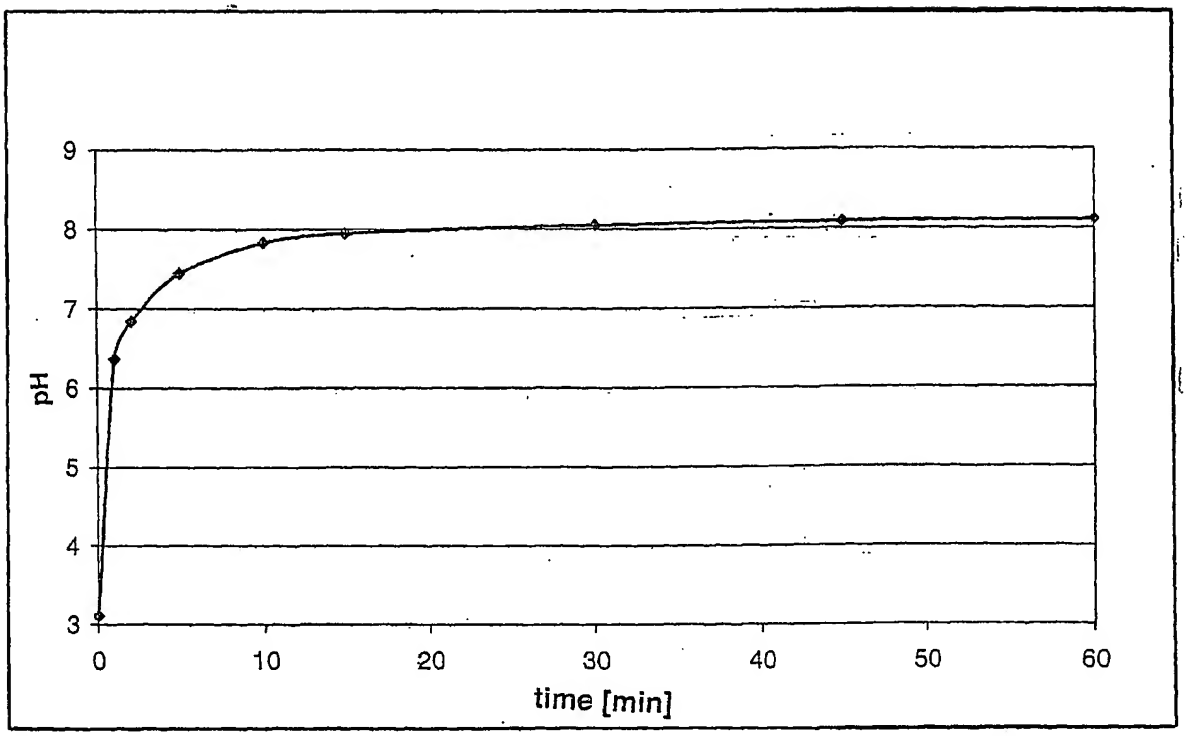

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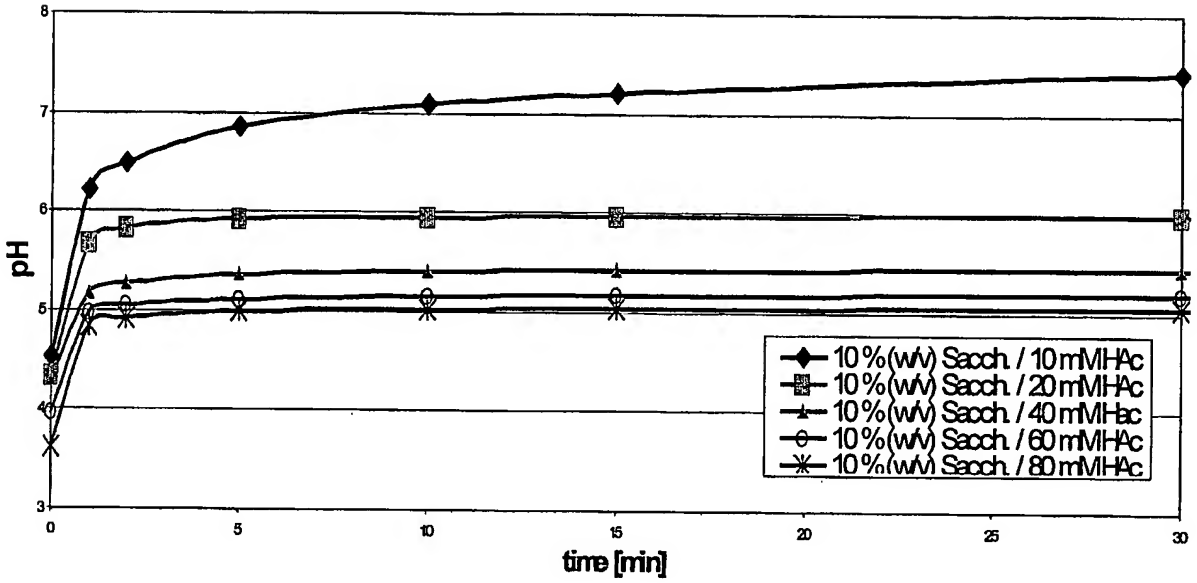
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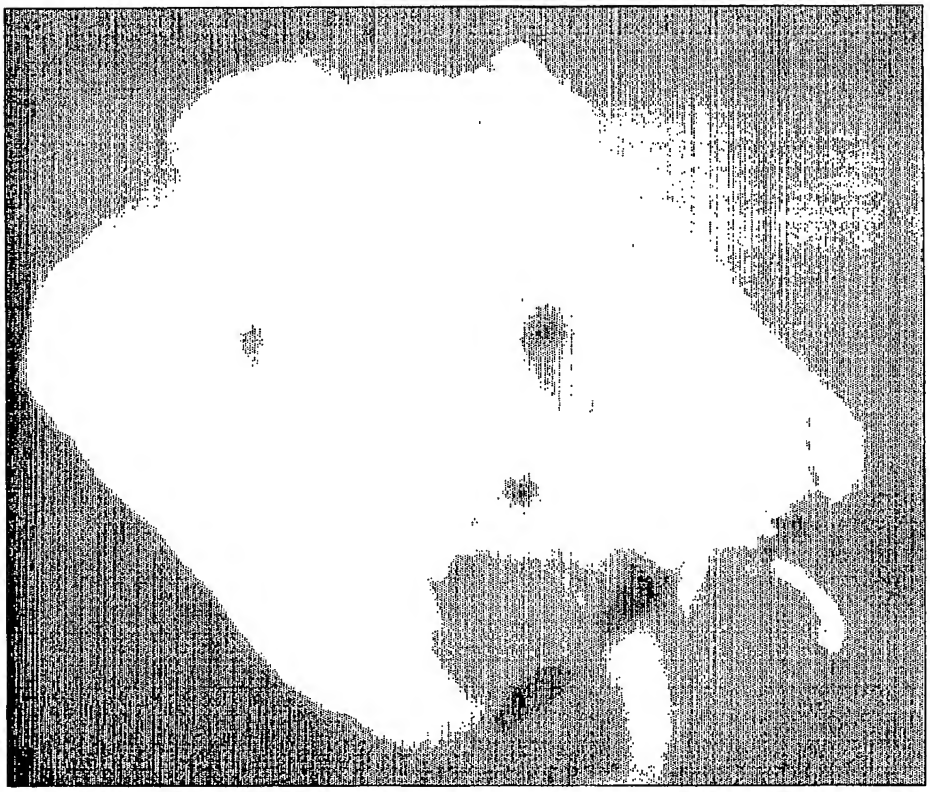
Figure 7

Figure 8



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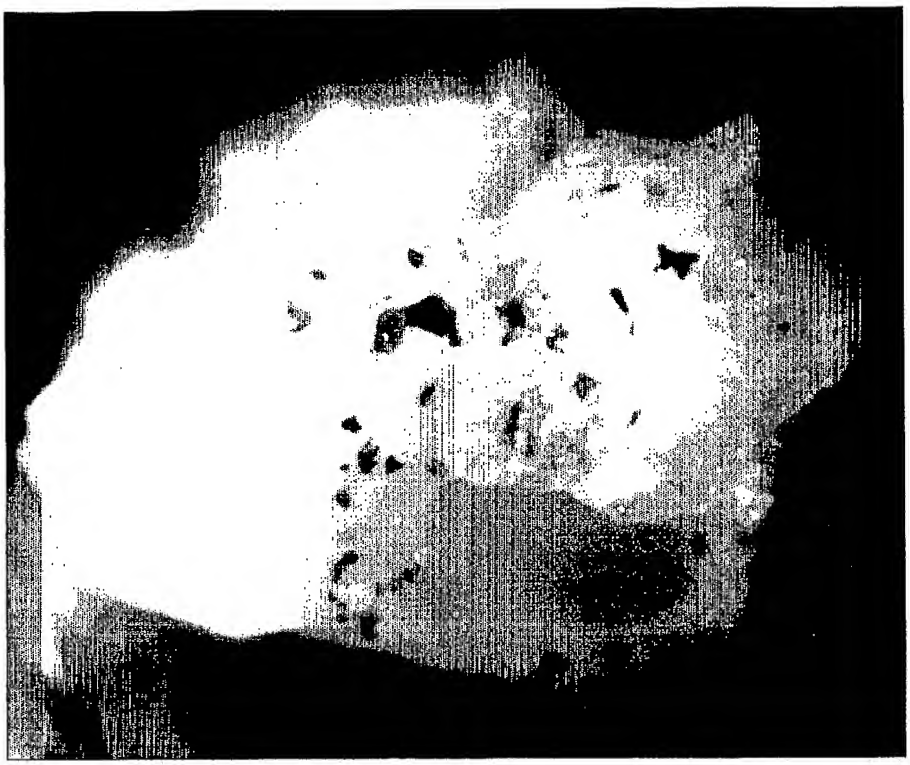
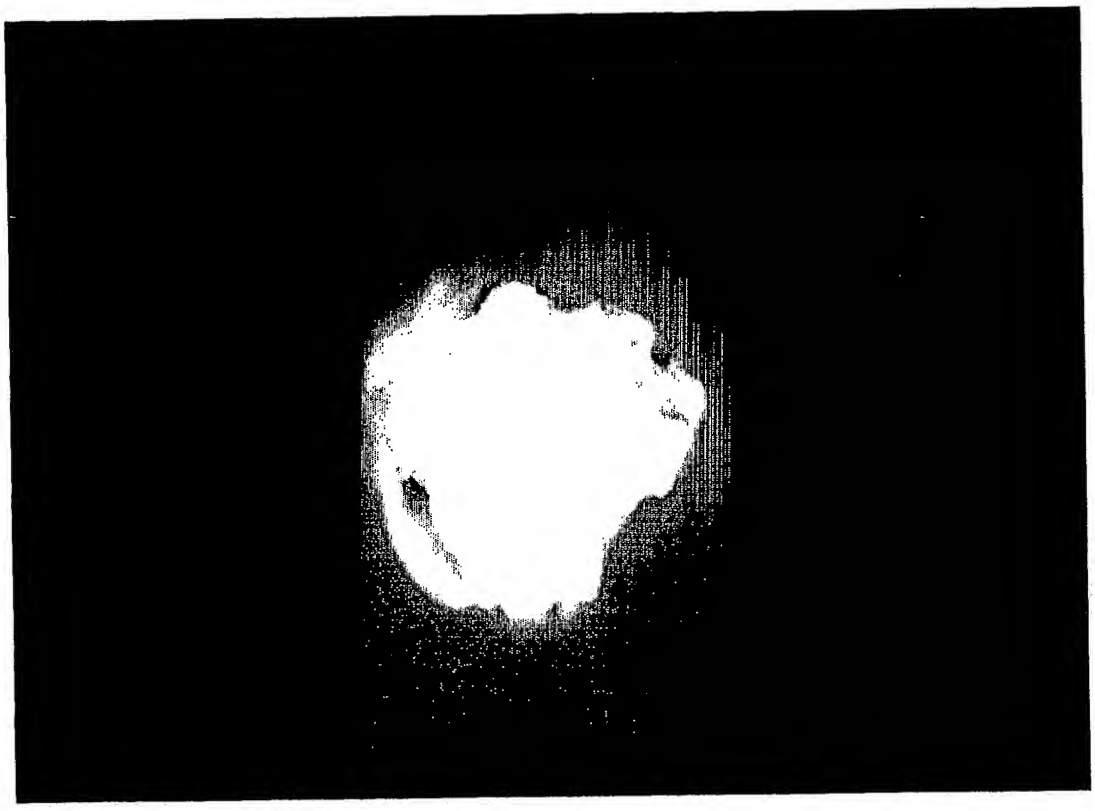


Figure 10

Figure 11

Figure 12



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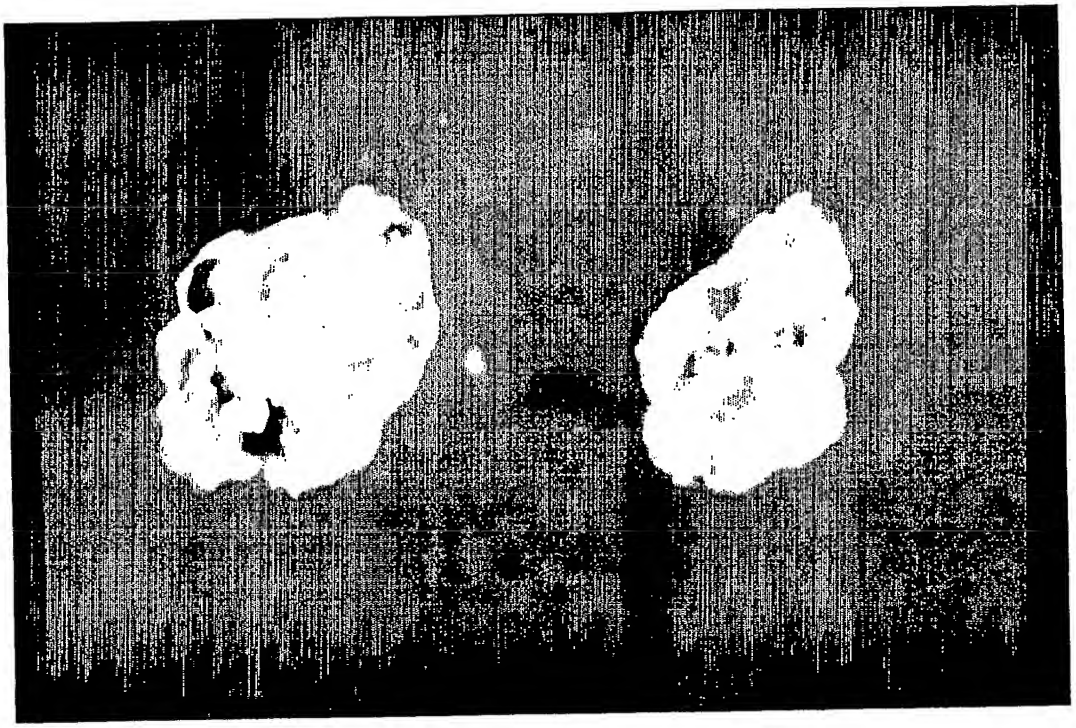

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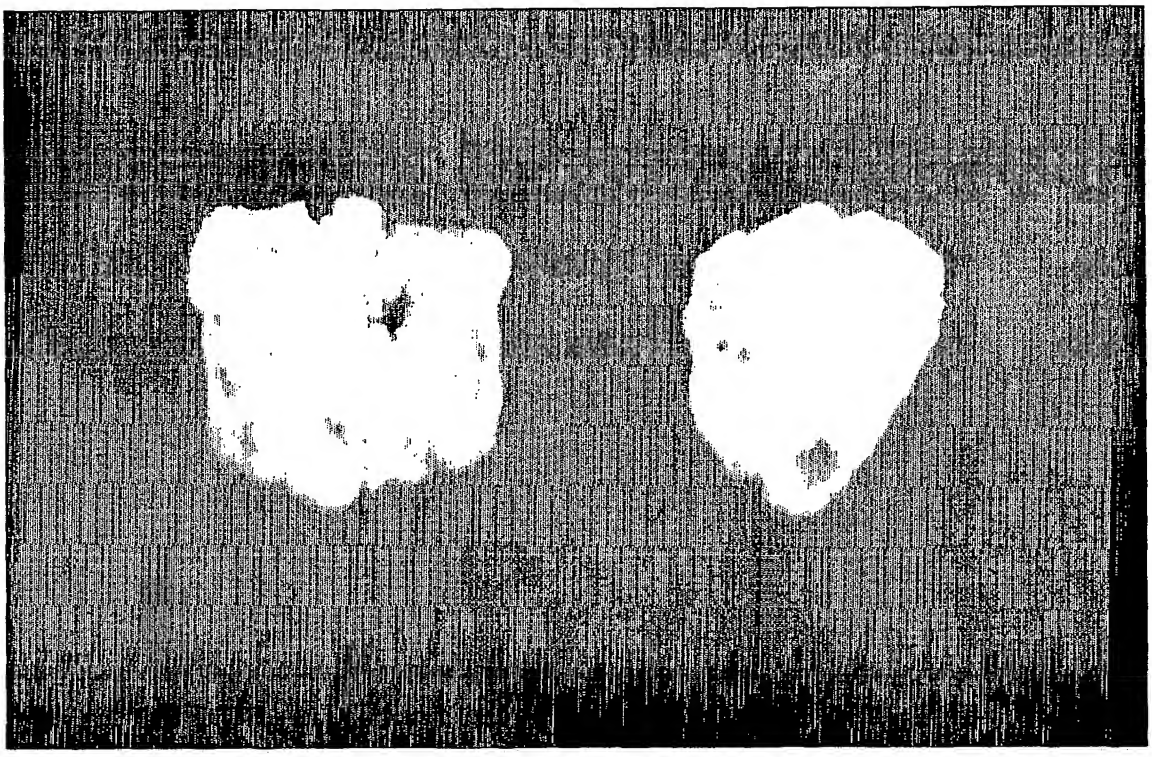

SEQUENCE LISTING

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<150> 01 12 7573.2
<151> 2001-11-19

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03463

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/34 A61L27/40 A61L27/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	DE 196 47 853 A (BIOPH BIOTECH ENTW PHARM GMBH ;GERONTOCARE GMBH BIOMATERIALS (DE)) 20 May 1998 (1998-05-20) the whole document ---	1,11-27 2-10
Y	US 5 385 887 A (HUBERTY MICHAEL C ET AL) 31 January 1995 (1995-01-31) column 2, line 31 - line 46 column 3, line 8 -column 5, line 2 column 8, line 29 -column 9, line 3; claim 1; example 4 ---	2-10
X	US 4 596 574 A (URIST MARSHALL R) 24 June 1986 (1986-06-24) column 4, line 14 - line 31; claims 1,5-8,15,16; example 1 --- -/--	1,11-23, 27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 December 2002	03/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Leutner, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03463

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 422 340 A (ONGPIPATTANAKUL BOONSRI ET AL) 6 June 1995 (1995-06-06) column 12, line 1 - line 19 column 14, line 24 - line 36; claims 1-4,16; examples 2,4,10	1,11-23, 27
X	WO 97 31661 A (LINDHOLM T SAM ;MATTINEN AULIS (FI)) 4 September 1997 (1997-09-04) page 4, paragraph 7 -page 5, paragraph 3 page 6, paragraph 1 - paragraph 4 page 8, paragraph 6; claims 1,10-15	1,11-23, 27
X	IMRANUL ALAM M ET AL: "Evaluation of ceramics composed of different hydroxyapatite to tricalcium phosphate ratios as carriers for rhBMP-2" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 22, no. 12, 15 June 2001 (2001-06-15), pages 1643-1651, XP004245902 ISSN: 0142-9612 the whole document	1-23,27
X	WO 00 72775 A (NOBEL BIOCARE AB (SE)) 7 December 2000 (2000-12-07) page 4, line 4 - line 37	1,11-23, 27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/03463

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
DE 19647853	A	20-05-1998	DE	19647853 A1	20-05-1998
			AU	5553398 A	10-06-1998
			WO	9821972 A2	28-05-1998
			EP	0942758 A2	22-09-1999
			JP	2001505097 T	17-04-2001
US 5385887	A	31-01-1995	AU	695374 B2	13-08-1998
			AU	7953794 A	27-03-1995
			EP	0724459 A1	07-08-1996
			FI	961037 A	06-03-1996
			JP	9502368 T	11-03-1997
			NO	960905 A	06-03-1996
			WO	9507108 A2	16-03-1995
US 4596574	A	24-06-1986	NONE		
US 5422340	A	06-06-1995	US	5158934 A	27-10-1992
			AT	153535 T	15-06-1997
			AU	671721 B2	05-09-1996
			AU	6026294 A	15-08-1994
			CA	2151486 A1	21-07-1994
			DE	69403439 D1	03-07-1997
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			DK	679097 T3	22-12-1997
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			ES	2105641 T3	16-10-1997
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			JP	8505548 T	18-06-1996
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			US	5409896 A	25-04-1995
			US	5604204 A	18-02-1997
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			AU	4721696 A	16-09-1997
			EP	0883410 A1	16-12-1998
			FI	981818 A	12-10-1998
WO 0072775	A	07-12-2000	SE	515695 C2	24-09-2001
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